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ATP-Dependent Chromatin Remodeling Is Required for Base Excision Repair in Conventional but Not in Variant H2A.Bbd Nucleosomes▽

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In eukaryotes, base excision repair (BER) is responsible for the repair of oxidatively generated lesions. The mechanism of BER on naked DNA substrates has been studied in detail, but how it operates on chromatin remains unclear. Here we have studied the mechanism of BER by introducing a single 8-oxo-7,8-dihydroguanine (8-oxoG) lesion in the DNA of reconstituted positioned conventional and histone variant H2A.Bbd nucleosomes. We found that 8-oxoguanine DNA glycosylase, apurinic/apyrimidinic endonuclease, and polymerase β activities were strongly reduced in both types of nucleosomes. In conventional nucleosomes SWI/SNF stimulated the processing of 8-oxoG by each one of the three BER repair factors to efficiencies similar to those for naked DNA. Interestingly, SWI/SNF-induced remodeling, but not mobilization of conventional nucleosomes, was required to achieve this effect. A very weak effect of SWI/SNF on the 8-oxoG BER removal in H2A.Bbd histone variant nucleosomes was observed. The possible implications of our data for the understanding of in vivo mechanisms of BER are discussed.

Eukaryotic cells are constantly subjected to oxidative stress. The reactive oxygen species generated during cell metabolism react with the nucleobases and damage DNA at any moment of the cell life. 8-Oxo-7,8-dihydroguanine (8-oxoG), the major reactive oxygen species-induced oxidative lesion, is repaired by the base excision repair (BER) pathway (27). The first step in the BER pathway is the recognition and the removal of 8-oxoG by 8-oxoguanine DNA glycosylase (OGG1), a bifunctional *N*-glycosylase which exhibits both a glycosylase and an apurinic/apyrimidinic (AP) lyase activity (38, 44). The DNA nicked by OGG1 at the site of the lesion (3′ to the lesion) is then processed by an *ap*urinic/*ap*yrimidinic *e*ndonuclease (APE1), which creates a free 3′-hydroxyl group required for the gap filling by polymerase β (Pol β). Finally, the 8-oxoG repair is completed by ligation of the nick by DNA ligase III (for a review, see reference 28).

DNA in the eukaryotic cell is packaged into chromatin. The first level of chromatin organization, the nucleosome, consists of an octamer of core histones (two each of H2A, H2B, H3, and H4) around which ~150 bp of DNA is wrapped into two superhelical turns (45). In addition to the conventional histones, eukaryotic cells express histone variants, which are incorporated into chromatin and form variant nucleosomes (45). Histone variants are nonallelic isoforms of conventional histones and show various degrees of homology to their conventional counterparts (39). The recently identified histone variant H2A.Bbd (*B*arr *b*ody *d*eficient), which exhibited only 48% identity with H2A, is found to be localized in transcriptionally active regions of the nucleus (8). H2A.Bbd nucleosomes have a more relaxed structure, they are less stable and more easily transcribed than conventional nucleosomes (14), and they are resistant to remodeling by SWI/SNF (3).

The repair proteins catalyzing the different steps in BER on naked DNA substrates have been studied in detail (13, 35). How BER acts on DNA organized into nucleosome, however, remains unclear, and the reported data exhibit some disagreements (5, 6, 25, 33). For example, in one study (33), uracil DNA glycosylase (UDG) showed 3- to 10-fold-reduced accessibility in nucleosomes independently on rotational setting, while in other studies (5, 6), a 10- to 30-fold inhibition of UDG by the nucleosome and a strong dependence of UDG processing on the rotation position of the uracil was reported. Interestingly, similar efficiencies in processing naked DNA and nucleosomes were reported for the FLAP endonuclease (FEN1) and DNA ligase I (9, 24), suggesting that these BER steps do not require nucleosome remodeling. Although a functional correlation between 8-oxoG repair and the Cockayne syndrome B protein was established in vivo (41, 42), direct in vitro studies on the action of ATP-dependent chromatin remodelers on BER were not reported.

We have introduced a single 8-oxoG lesion in the vicinity of the dyad axis of conventional and variant H2A.Bbd nucleosomes and have studied their repair. We show that the efficiency of BER was dramatically reduced in both types of nucleosomes. In addition, we present evidence that the 8-oxoG

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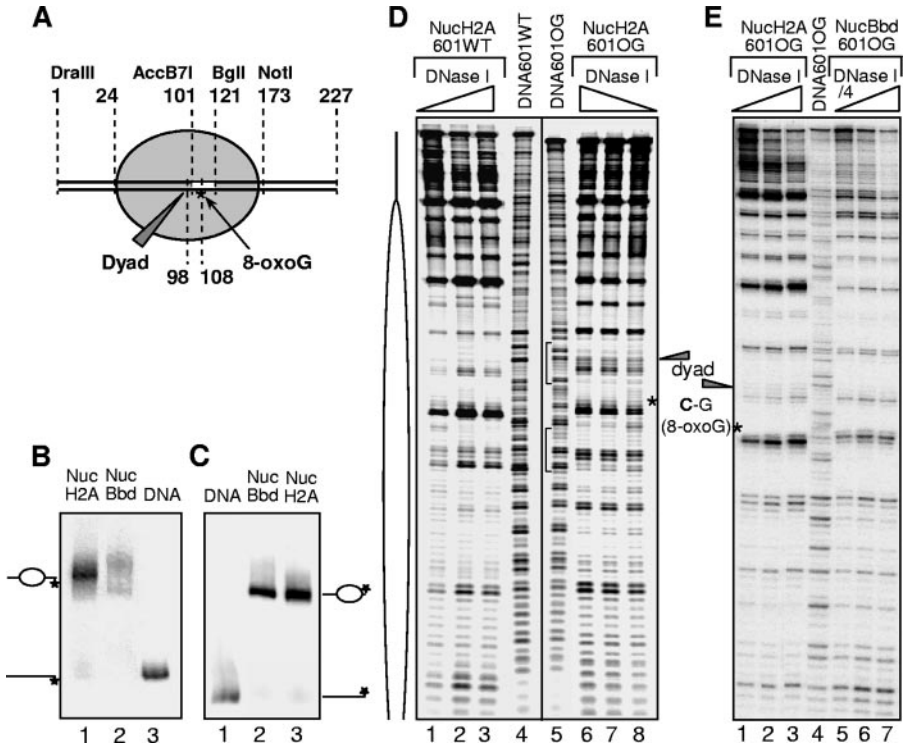

FIG. 1. EMSA and DNase I footprinting analysis of reconstituted conventional (NucH2A) and variant (NucBbd) nucleosomes on 601WT and 601OG DNA fragments. (A) Schematic representation of the positioned histone octamer on the 601OG DNA. The positions of 8-oxoG, the cleavage sites for restriction enzymes (numbered in the 5′ to 3′direction of the sense strand), and the dyad axis are indicated. (B) EMSA in 5% polyacrylamide gels of the 5′-labeled reconstituted conventional (lane 1) and variant (lane 2) 227-bp 601 nucleosomes used in repair assays. The slightly different migration of the nucleosomes containing H2A.Bbd is likely due to a difference in the conformation of linker DNA. The 5′-^{32}P label position (bottom strand) is indicated by an asterisk (see Materials and Methods). (C) EMSA of the end-positioned conventional (lane 3) and variant (lane 2) nucleosomes, reconstituted on NotI-restricted and 3′-labeled 601 DNA (upper strand) used in DNase I footprinting experiments. The 3′-^{32}P label position is indicated by an asterisk (see Materials and Methods). (D) DNase I footprinting analysis of conventional (NucH2A) nucleosomes reconstituted with NotI-cleaved 601WT (lanes 1 to 3) and 601OG (lanes 6 to 8) DNAs. The brackets indicate the positions of the inserted AccB7I and BglI sites. The typical 10-bp repeat is present in both the 601WT and the modified 601OG-positioned nucleosomes. (E) DNase I footprinting of conventional (NucH2A) (lanes 1 to 3) and variant (NucBbd) (lanes 5 to 7) nucleosomes reconstituted with the NotI restriction 601OG DNA fragment. Note the higher accessibility of the NucBbd nucleosomes to DNase I. The positions of the dyad and the cytosine, complementary to 8-oxoG, are indicated.

repair of conventional, but not H2A.Bbd, nucleosomes was greatly facilitated by the chromatin remodeler SWI/SNF. This effect did not depend on the SWI/SNF-mediated mobilization status of the nucleosomes.

MATERIALS AND METHODS

DNA probes. Plasmid pGEM-3Z-601 (a kind gift from J. Widom and B. Bartholomew), containing the strong nucleosome positioning wild-type sequence 601WT (29), was used as a template for the cloning of nonpalindromic restriction sites AccB7I and BglI. The oligonucleotides and complete sequences used for this study are available as supplementary data S1 at http://www.ens-lyon.fr/Joliot-Curie/fichiers/supdata_menoni_et_al_S1.pdf. Briefly, the restriction sites DraIII, AccB7I, and BglI were introduced at positions 18, 119, and 139 of the original sequence, respectively. This new construct was cloned in a pGEM-T vector and further used as a template to produce the 601OG. The solid-phase synthesis of the modified oligonucleotide was performed using an Applied Biosystems 392 DNA synthesizer, using 8-oxodG phosphoramidite monomer as previously described (7). Both the modified and complementary oligonucleotides were purified by high-pressure liquid chromatography, and then matrix-assisted laser desorption ionization–time-of-flight characterization showed more than 90% mass homogeneity. To introduce the single 8-oxoG near the dyad of the nucleosome, we produced the full-length fragment 601OG by PCR and digested the fragment with AccB7I and BglI. The 20-mer fragments released by digestion were removed by two purifications on a High Pure PCR purification column (Roche). The insertion of 8-oxoG was realized by ligation of the two larger fragments (121 and 106 bp) with the double-stranded 20-mer carrying 8-oxoG, and the resulting 245-bp DNA fragment was gel purified. For the repair experiment, the 601OG was cut with DraIII and labeled with polynucleotide kinase and [γ-^{32}P]ATP on the strand carrying 8-oxoG (Fig. 1A). For DNase I footprinting experiments, the 601WT and the 601OG DNAs were cut with NotI and the strand complementary to the 8-oxoG strand was labeled with Klenow enzyme with [α-^{32}P]CTP in the presence of 50 μM dGTP and gel purified.

Proteins. Conventional recombinant *Xenopus laevis* full-length histone proteins (30) and the variant H2A.Bbd histone (3) were produced in bacteria and purified as described previously. Yeast SWI/SNF complex was expressed and purified as described previously (12). The recombinant murine enzyme OGG1 was purchased from Sigma-Aldrich. According to the producer, one unit of enzyme cleaves 50% of 0.5 pmol of DNA substrate in 10 min at 37°C. Human APE1 and DNA Pol β were purchased from Trevigen. One unit of APE1 processes an AP site in DNA substrates at the rate of 1 pmol/h at 37°C. One unit of DNA Pol β catalyzes the incorporation of 1 nmol of deoxynucleoside triphosphate into the acid-soluble form in 1 h at 37°C.

Reconstitution and characterization of positioned nucleosomes. Nucleosomes were reconstituted by salt dialysis using ~50 ng of the labeled DNA probe, 2.5 μg of nucleosomal size (or plasmid for the Pol β experiments) carrier DNA (1/50 ratio), and recombinant histones (2, 3, 32). The appropriate histone/DNA ratio was carefully adjusted experimentally in order to reduce the content of free DNA in all nucleosomal preparations to less than 3 to 4%. Electrophoretic mobility shift assay (EMSA) was carried out in 5% (wt/vol) polyacrylamide gels (29:1) and

0.3× Tris-borate-EDTA. For DNase I footprinting experiments, end-positioned nucleosomes reconstituted on NotI-cleaved and 3′-labeled 601OG and 601WT DNA fragments were used. The experiments were performed as described previously (2, 3). The ratio of the amounts of DNase I used for conventional and Bbd nucleosomes and free DNA was 1:0.25:0.05.

Assays for OGG1, APE1, Pol β, and SWI/SNF activities. Each of the repair enzymes was incubated with 0.5 pmol of nucleosomal substrate, containing ~10 fmol of 8-oxoG-modified labeled DNA probe, either naked or nucleosome reconstituted, in a repair buffer consisting of 10 mM Tris-HCl (pH 7.4), 32 mM NaCl, 3.5 mM $MgCl_2$, 200 μM EDTA, 8 mM KCl, 100 μg/ml bovine serum albumin, 1 mM dithiothreitol, 0.02% NP-40, and 5% glycerol. The quantification was carried out by normalizing the amount of OGG1-cleaved DNA to the maximum 75% cleaved DNA. Where indicated, SWI/SNF and 1 mM ATP were added to the reaction mixture. One unit of SWI/SNF is the amount (approximately 10 fmol) necessary to induce 50% sliding of 0.5 pmol of middle-positioned nucleosomes. To maintain the integrity of nucleosomes, which at 37°C is likely to be compromised by thermal DNA unwrapping and/or octamer sliding (16, 17), all reactions were carried out at 29°C. Incubation times were selected in order to achieve full saturation of the respective activities as determined by kinetic experiments. Reactions were stopped with 20 mM EDTA–0.1% sodium dodecyl sulfate, and DNA was extracted with phenol-chloroform and ethanol precipitated prior to analysis by 8% acrylamide–8 M urea denaturating gel electrophoresis. Nucleosomes used in Pol β experiments were reconstituted on 601OGG1-APE1 DNA substrate, obtained by OGG1 and APE1 processing of 601OG DNA. These nucleosomes were incubated for 60 min with 0.1 unit of Pol β and 0.05 μCi of [^{32}P-α]dGTP in the presence or absence of SWI/SNF (see Fig. 6A).

RESULTS

Characterization of conventional and H2A.Bbd nucleosomes carrying a single 8-oxoG. In order to study repair in a nucleosomal template, we first reconstituted a nucleosome containing a single 8-oxoG in the vicinity of the dyad axis. Briefly, in the strong-positioning 601 DNA sequence (29), we inserted two restriction sites for the enzymes AccB7I and BglI next to the dyad (Fig. 1A) (see supplementary data S1 at http://www.ens-lyon.fr/Joliot-Curie/fichiers/supdata_menoni_et_al_S1.pdf). Then, a chemically synthesized oligonucleotide carrying the 8-oxoG residue was introduced between these restriction sites by ligation. The full-length 227-bp nucleosome and the NotI-restricted wild-type (601WT) and modified (601OG) DNA fragments were uniquely 5′ or 3′ end labeled and used to reconstitute both conventional (NucH2A) and variant (NucBbd) nucleosomes (Fig. 1B and C). In the 227-bp nucleosomes used in the repair assay, the 8-oxoG lesion was located at position 108 relative to the 5′ end of the fragment and thus, at 10 bp from the dyad axis, was situated at position 98. Position 108 is located exactly between the most and the least exposed DNA sites within the nucleosome (Fig. 1A) (see Materials and Methods and supplementary data S1 at http://www.ens-lyon.fr/Joliot-Curie/fichiers/supdata_menoni_et_al_S1.pdf for details). Importantly, under our experimental conditions for nucleosome reconstitution, only very small amounts (less than 3 to 4%) of the available DNA remained free (Fig. 1B and C). The conventional (Fig. 1D and E) and the variant (Fig. 1E) nucleosomes, reconstituted on either the 601WT or the 601OG sequence, presented a very clear 10-bp repeat. A higher rate of DNase I digestion of Bbd nucleosomal DNA compared to that of conventional nucleosomes was observed. Some differences in the DNase I digestion patterns of the two nucleosomes were also detected (compare lanes 1 to 3 with lanes 5 to 7 in Fig. 1E). We attributed these effects to the less stable and more open structure of the H2A.Bbd nucleosome (3, 4, 14). Only negligible differences arising from the nucleotide substitution, however, were found in the DNase I cleavage pattern of naked DNA (Fig. 1D, compare lane 4 with lane 5). These data demonstrate that neither the presence of both 8-oxoG and the insertion of AccB7I and BglI restriction sites nor the variant histone affects the translational and rotational positioning of the histone octamer, a requirement for a well-defined study on a homogeneous nucleosomal template.

Conventional and H2A.Bbd octamers strongly inhibit the activities of OGG1 and APE1. The glycosylase/AP lyase activities of OGG1 produce a nick in the DNA strand carrying 8-oxoG, and this can be visualized by electrophoresis under denaturing conditions since the full-length DNA strand is 227 nucleotides, while the cleaved DNA strand is 119 nucleotides, in length (Fig. 2A). This allowed us to calculate the cleavage yield (the ratio of the intensity of the cut band to the sum of intensities of the cut and uncut bands, normalized to the maximum 75% fraction of cleavable DNA [see Materials and Methods for details]) for both naked and nucleosomal (NucH2A) DNAs at increased concentrations of OGG1 (Fig. 2B). The data show that the zero-dilution-extrapolated cleavage efficiency (the initial slope of the curve) of naked DNA by OGG1 was 92- ± 18-fold higher than that of the NucH2A nucleosome (Fig. 2C). The presence of the variant octamer also interfered with the OGG1 cleavage, but to a lesser extent than the conventional octamer (Fig. 2D). The initial efficiency of OGG1 cleavage was ~4- to 5-fold higher in the H2A.Bbd nucleosome than in the conventional one. In addition, for both nucleosomes, the extrapolated asymptotic values for the maximum cleavage were 80% ± 10%, which is in accordance with reported data (6).

After the removal of 8-oxoG and the induction of a nick at the site of damage, the next step of BER is the generation by APE1 of a 3′OH extremity at the AP site produced by OGG1. To test if this step was affected by the presence of a histone octamer, we first produced a well-detectable OGG1-processed DNA band by 90 min of treatment with 0.1, 0.5, and 0.25 unit of OGG1 with naked 601OG DNA and conventional and H2A.Bbd nucleosomes, respectively. Then, either 0.2 or 1 unit of APE1 was added, and the reaction mixture was further incubated for an additional 60 min. It is noteworthy that the AP lyase activity of OGG1 is slower than its *N*-glycosylase activity, which might result in additional DNA cleavage by APE1 (23, 46). Under our experimental conditions, however, we did not detect any additional DNA strand cleavage induced by APE1. The OGG1-APE1-processed band was discriminated from the OGG1 cleavage band through its faster migration on the DNA sequencing gel. Upon incubation with 0.2 unit of APE1, more than 50% of the naked DNA OGG1-cleaved band was processed by APE1 (Fig. 3A, lane 4). In contrast, the estimated amounts of conventional and variant nucleosomal DNAs processed by APE1 were about 10% and 17%, respectively (Fig. 3B and C, lanes 4). Moreover, 1 unit of APE1 was able to process the entire available naked DNA and only ~30% and 45% of the nucleosomal H2A and H2A.Bbd DNAs, respectively (Fig. 3B and C, lanes 5, and results not shown). Therefore, both the conventional and H2A.Bbd histone octamers interfere with the function of OGG1 and APE1.

SWI/SNF stimulates 8-oxoG BER of conventional but not of variant H2A.Bbd nucleosomes. The presence of the histone octamer interferes with 8-oxoG processing by both OGG1 and

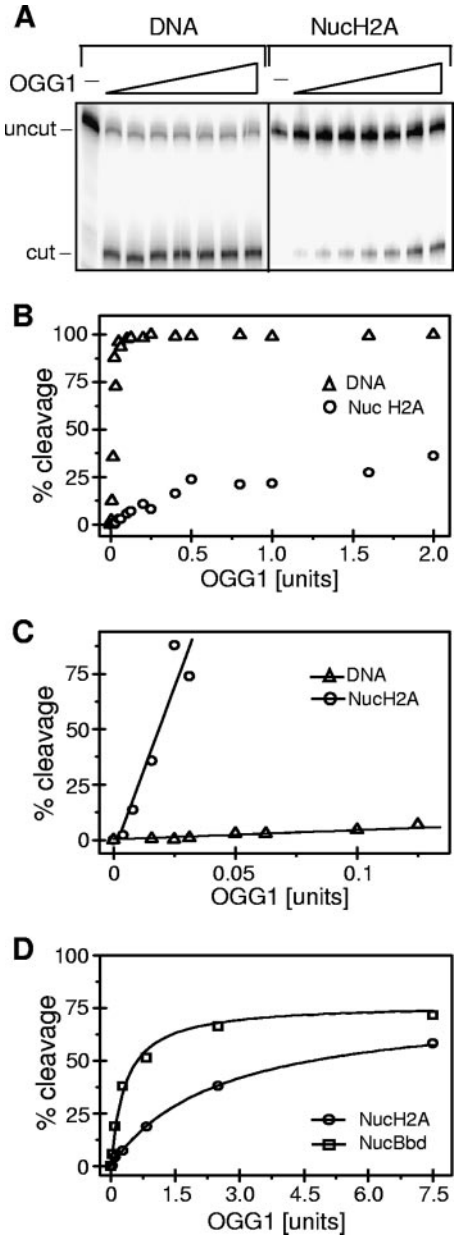

FIG. 2. Inhibition of the glycosylase/AP lyase activity of OGG1 and APE1 by conventional and variant H2A.Bbd nucleosome. (A) Equal amounts of either naked 227-bp 601OG DNA or reconstituted conventional nucleosomes were digested for 90 min at 29°C with increasing amounts of OGG1. The products were then resolved by 8% PAGE under denaturing conditions. (B) Quantification of the gels in panel A. The cleavage yield (percent cleavage) was calculated as the ratio of the intensity of the cleaved DNA band to the sum of intensities of the cleaved and the uncleaved DNA bands. For easier comparison, cleavage yields were normalized to the maximal 75% fraction of OGG1-cleavable naked DNA substrate. Data points were averaged over four independent experiments. (C) Data for low OGG1 concentration in panel B, interpolated by a linear function. (D) Comparison of the cleavage efficiencies in conventional (NucH2A) and variant (NucBbd) nucleosomes. Note that the interpolated asymptotic value at high OGG1 concentration for both curves is ~80% ± 10%.

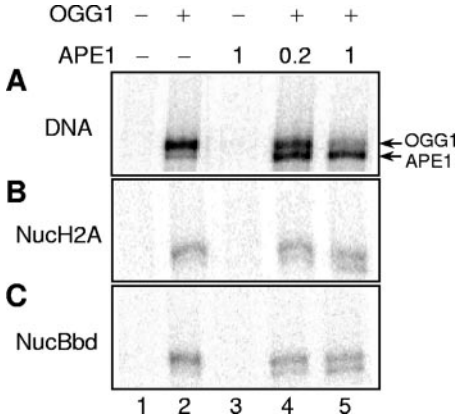

FIG. 3. The activity of APE1 is strongly reduced in the presence of the histone octamer. Sequencing gel analysis of the OGG1-cleaved and APE1-processed naked DNA (A) and the conventional NucH2A (B) and NucBbd variant (C) nucleosomal DNAs. Naked DNA and the conventional H2A and variant Bbd nucleosomes were first incubated for 90 min with 0.1, 0.5, and 0.25 unit of OGG1 (lanes 2, 4, and 5), respectively, and then 0.2 or 1 unit of APE1 (lanes 4 and 5) was added and the reaction mixtures were incubated for an additional 60 min. The purified DNA was run on an 8% denaturing polyacrylamide gel. The unsaturated sugar residue at the 3′ end still present in the OGG1-cleaved band is removed by APE1, allowing discrimination of their respective activities by the difference in gel migration.

APE1 BER enzymes. The nucleotide excision repair (NER) pathway was also inhibited by the nucleosome, but the inhibition was moderately relieved by SWI/SNF (21, 22). This prompted us to test whether the BER of 8-oxoG could also be assisted by the nucleosome-remodeling factor SWI/SNF (Fig. 4). Naked DNA, conventional and H2A.Bbd nucleosomes were subjected to the simultaneous action of OGG1 (0.3 unit) and APE1 (0.5 unit) in the presence of 2 units of SWI/SNF. These amounts of OGG1 and APE1 were chosen since they allowed the generation of detectable processed DNA bands in all three samples. Incubation of 0.5 pmol of conventional nucleosomes with increasing amounts of SWI/SNF resulted in an increasing loss of the DNase I 10-bp repeat (Fig. 4A, lanes 1 to 4), providing evidence for perturbation of the histone-DNA interactions within conventional nucleosomes. Under these conditions, SWI/SNF was unable to perturb the structure of the H2A.Bbd nucleosome (Fig. 4A, lanes 6 to 9), since only a very slight remodeling of Bbd nucleosomes at the highest level of SWI/SNF used was detected (Fig. 4A, lane 6), a result in agreement with our previous data (3, 14).

As expected, SWI/SNF did not increase the processing of naked DNA but had a small inhibitory effect, probably due to some unspecific binding of the remodeling complex to the DNA (Fig. 4B, lanes 1 and 2, and C). SWI/SNF had only a very small (not exceeding 1.5-fold) effect on the H2A.Bbd nucleosome processing (Fig. 4B, lanes 5 and 6, and C), which is not surprising since this nucleosome is refractory to the action of SWI/SNF. In contrast, SWI/SNF treatment of the conventional nucleosome induced a dramatic increase (to a level similar to that of naked DNA) of the 8-oxoG processing by OGG1 and APE1, (Fig. 4B, lanes 3 and 4, and C). We conclude that SWI/SNF is required for the repair of 8-oxoG in conventional nucleosomes.

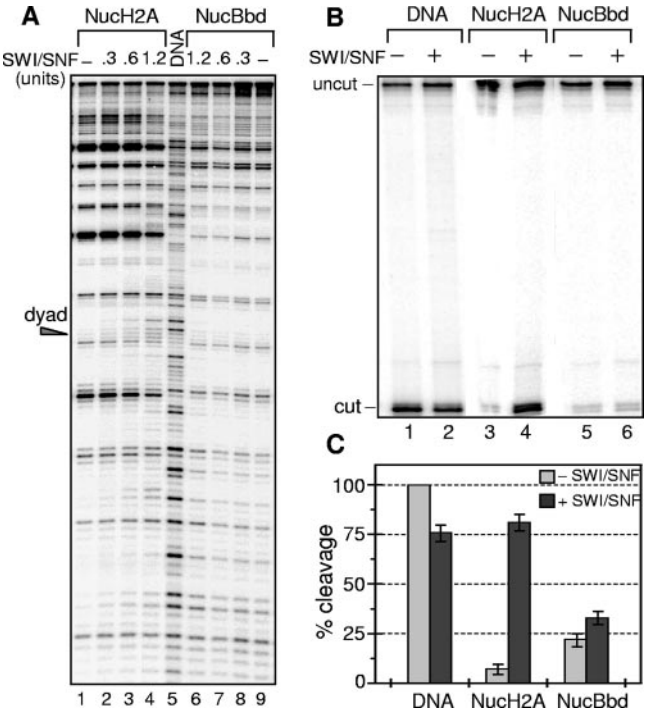

FIG. 4. SWI/SNF drastically increases the activity of OGG1 and APE1 on conventional nucleosomes but has little effect on nucleosomes containing H2A.Bbd. (A) DNase I footprinting analysis of SWI/SNF-treated nucleosomes. Conventional (lanes 1 to 4) and variant (lanes 5 to 9) nucleosomes were incubated with various amounts of SWI/SNF as indicated for 45 min and then digested with DNase I. DNA isolated from control (lane 5) and SWI/SNF-treated nucleosomes were run on an 8% denaturing polyacrylamide gel. (B) Naked 601OG DNA (lanes 1 and 2) and conventional (lanes 3 and 4) and variant H2A.Bbd (lanes 5 and 6) nucleosomes were incubated for 90 min simultaneously with 0.2 unit of OGG1, 1 unit of APE1, and 2 units of SWI/SNF (lanes 2, 4, and 6). The purified DNA was run on an 8% denaturing polyacrylamide gel. (C) Quantification of the data presented in panel B. The means value and standard deviations from three independent experiments are shown.

The effect of SWI/SNF on a conventional nucleosome is dual: SWI/SNF is able to perturb the histone-DNA interactions (remodeling of the nucleosome) and to mobilize the histone octamer (sliding of the octamer). Is SWI/SNF assisting BER of 8-oxoG through a nucleosome remodeling mechanism or through a histone octamer sliding mechanism? To differentiate between these two possibilities, we carried out the experiments schematically depicted in Fig. 5A. We incubated the centrally positioned conventional nucleosomes for 90 min with either 0.5 or 1 unit of SWI/SNF in the presence of a limited amount (0.1 or 0.2 unit) of OGG1. The reaction mixture was run on a native polyacrylamide gel to separate the SWI/SNF-mobilized (end-positioned) nucleosomes from the centrally positioned ones (Fig. 5B). Then, the bands corresponding to the two types of nucleosomes were excised from the gel, and DNA was eluted and analyzed by polyacrylamide gel electrophoresis (PAGE) under denaturing conditions. The efficiency of OGG1 cleavage was subsequently measured (Fig. 5C). The data clearly showed that both the centrally (nonmobilized) and the end-positioned (mobilized) nucleosomes exhibited enhanced 8-oxoG accessibility. Interestingly, the accessibility of the relocated end-positioned nucleosomes was only 25 to 30% higher than that of the nonmobilized ones (Fig. 5C). We conclude that the ATP-dependent SWI/SNF-induced accessibility of 8-oxoG to OGG1 reflects mainly the SWI/SNF-generated structural alterations in the nucleosomes and not their relocation.

The experiments described above were informative about the changes in the cleavage efficiency of OGG1 during the action of SWI/SNF on the nucleosome. However, SWI/SNF induces perturbations in the nucleosome structure, which persist after arresting SWI/SNF (11). To study the effect of these persistent nucleosomal alterations, we carried out similar experiments, but the nucleosomes were first incubated with SWI/SNF, then the reaction was arrested with apyrase, and the nucleosomes were subsequently treated with OGG1 (Fig. 5A). The results (Fig. 5D) were very close to those obtained when the experiment was performed in the presence of active SWI/SNF, further confirming that the changes in the enhanced OGG1 processing of 8-oxoG were associated mainly with the nucleosome remodeling.

Pol β requires SWI/SNF to synthesize DNA on nucleosomal templates. Pol β catalyzes the incorporation of the proper nucleotide opposite to the undamaged strand and the removal of the sugar phosphate moiety of the damaged base (31, 37). The strand elongation by Pol β was shown to interfere with the presence of a conventional histone octamer (5, 6, 33). The effect of nucleosome remodeling and histone variants on the function of Pol β has not been addressed in the literature. We have studied these questions by using nucleosomes reconstituted with either conventional or H2A.Bbd histone octamers on the 227-bp 8-oxoG 601 DNA fragment processed by both OGG1 and APE1 (Fig. 6A). The presence of the histone octamer strongly inhibited Pol β-dependent [α-^{32}P]GTP incorporation (Fig. 6B, compare lanes 2, 4, and 7), a result in agreement with the literature data (5, 6). The H2A.Bbd histone octamer exhibited a weaker inhibition effect (Fig. 6B, compare lanes 4 and 7). Treatment with SWI/SNF did not lead to a significant enhancement of [α-^{32}P]GTP incorporation into H2A.Bbd nucleosomes (Fig. 6B, lanes 7 and 8), while substantial SWI/SNF-dependent incorporation was detected in the conventional H2A nucleosome (Fig. 6B, lanes 4 and 5). This demonstrates that nucleosome remodeling would be a necessary step for the function of Pol β on conventional nucleosomal templates.

DISCUSSION

In this work we have shown that both conventional and variant H2A.Bbd nucleosomes strongly interfere with the first three steps of 8-oxoG repair mediated by OGG1, APE1, and Pol β. The initial rate of DNA cleavage at 8-oxoG by OGG1 was two orders of magnitude lower in conventional nucleosomal templates than in naked DNA. Interestingly, the nucleosome inhibition of OGG1 cleavage that we found exceeded by 3 to 30 times that reported for the UDG-APE1 processing (5, 6, 33). This difference might be explained by differences either in lesion-processing mechanisms of OGG1 and UDG or in the position of the lesion and partial heterogeneity of nucleosomes reconstituted on weaker-positioning sequences. The experimental difficulties in determining the initial rates of cleavage,

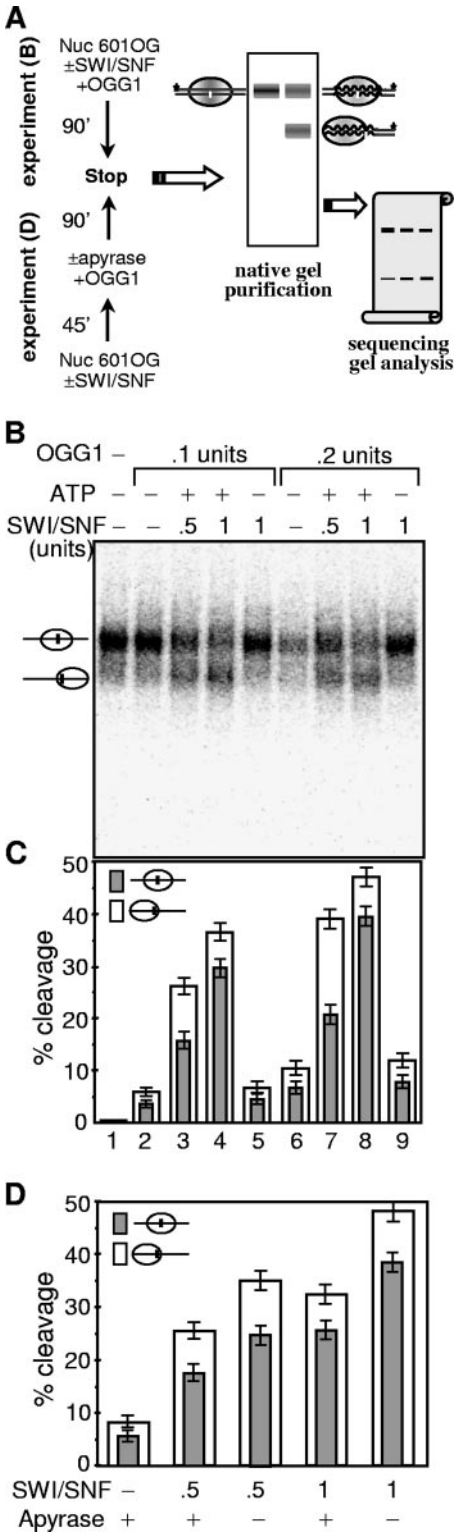

FIG. 5. Nucleosome remodeling by SWI/SNF increases 8-oxoG accessibility to OGG1 independently of the mobilization status of the nucleosome. (A) Schematics of the experiments on the role of SWI/SNF in 8-oxoG processing by OGG1. (B) Preparative EMSA used for analysis of OGG1 cleavage efficiency during the SWI/SNF treatment of conventional nucleosomes (see panel A for details). The locations of the centrally and end-positioned (relocated) nucleosomes are indicated. (C) Quantification of the data presented in panel B. (D) The experiments were carried out as for panel B, but SWI/SNF remodeling was stopped with apyrase prior to the addition of 0.2 unit of OGG1 (see panel A for detail). For panels C and D, the means and standard deviations from two independent experiments are presented.

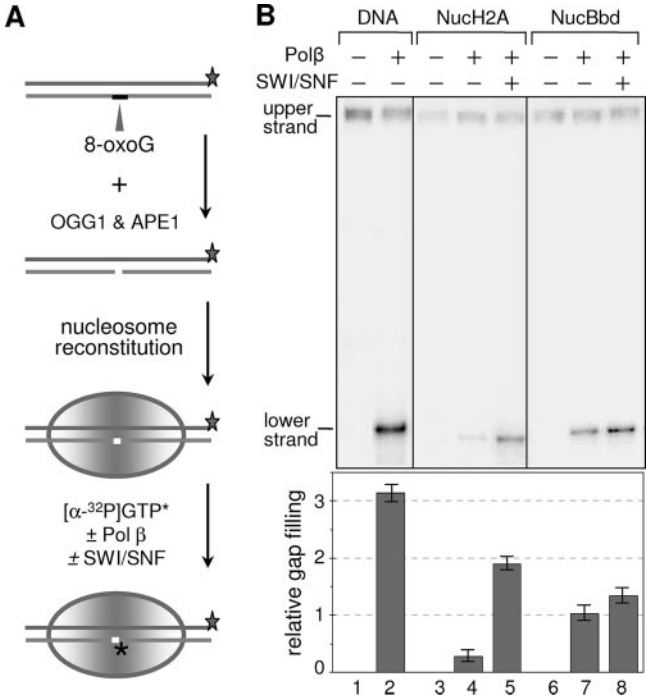

FIG. 6. SWI/SNF promotes the addition of a nucleotide by Pol β in conventional nucleosomes and has little effect on Bbd nucleosomes. (A) Schematics of the preparation of 601OGG1-APE1 nucleosomes and the Pol β experiment. ^{32}P-end-labeled 601OG DNA was first treated with OGG1 and APE1 and then used for nucleosome reconstitution and Pol β gap filling. (B) Naked DNA (lanes 1 and 2) and conventional (lanes 3 to 5) and variant (lanes 6 to 8) nucleosomes were incubated for 45 min with [α-^{32}P]GTP and Pol β (lanes 2, 4, 5, 7, and 8) in the absence (lanes 1 to 4, 6, and 7) or presence (lanes 5 and 8) of 1 unit of SWI/SNF. After purification, the processed DNA samples were separated by 8% denaturing PAGE. The lower panel shows the quantification of the data. The means and standard deviations from two independent experiments are presented.

requiring naked-DNA-free nucleosomes, could also interfere with the accurate measurement of the BER efficiency on nucleosomal templates.

At present, we cannot judge how the position of the lesion could affect the efficiency of 8-oxoG repair, since we have carried out experiments with 8-oxoG inserted in only a single position close to the dyad. Beard et al. (6) have shown, however, that the rate of the glycosylase-AP endonuclease activity of UDG-APE1 differed by 2 to 3 times between the most and the least exposed DNA sites closest to the dyad. With this in mind, we believed that there would be a similar dependence of the 8-oxoG repair on the orientation of the lesion relative to the solution.

APE1 and the Pol β functioned on nucleosomal templates very similarly to OGG1. The smaller inhibition of OGG1 by the H2A.Bbd nucleosome (~18- to 23-fold) relative to naked

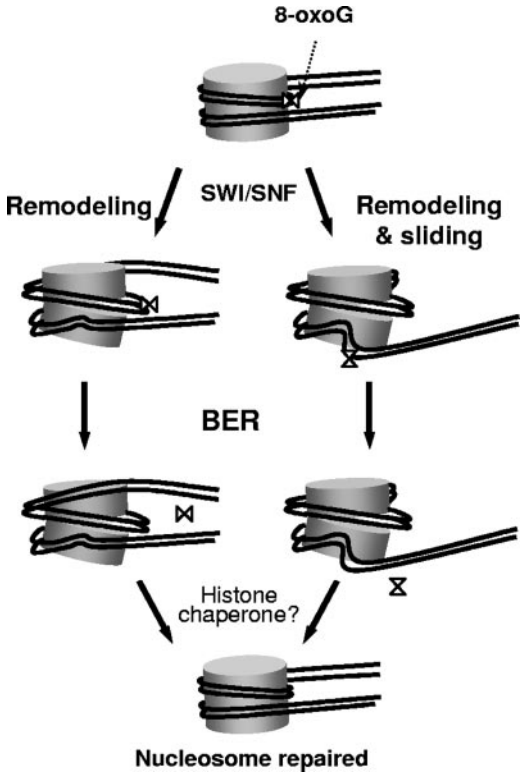

FIG. 7. Model for the BER of 8-oxoG in conventional nucleosomes.

DNA was not surprising, since previous studies have shown that this variant nucleosome was less stable and had a more open structure than the conventional nucleosome and that H2A.Bbd-H2B dimers were more mobile (3, 14, 18).

In the presence of SWI/SNF, the very strong inhibition of BER (OGG1, APE1, and Pol β activities) on the conventional nucleosome was relieved, since the efficiency of 8-oxoG repair was retrieved at a level close to that obtained with naked DNA. Notably, SWI/SNF was also able to assist NER, but its effect on NER was found to be very modest (a 1.5- to 2-fold increase of NER efficiency was reported in the presence of SWI/SNF) (21, 22). Therefore, our data demonstrate a qualitatively different effect of SWI/SNF on BER compared to NER and suggest that in vivo chromatin remodelers of the SWI/SNF type will be required for efficient BER of oxidative lesions.

Interestingly, the NER removal of a pyrimidine (6-4) pyrimidone photoadduct remained refractory to remodeling by ACF when the DNA lesion was inside the nucleosome, while the DNA lesion within the linker DNA was 2 times more efficiently repaired (43). The different effects of SWI/SNF and ACF on NER most probably result from the different remodeling mechanisms of these two enzymes (15). Recent in vivo data have also revealed correlations between the remodelers RSC and SWI/SNF with DNA double-strand breaks and UV photoproduct repair by NER (10, 26, 34). Bearing this as well as the results of the present work in mind, we predict that chromatin remodelers such as SWI/SNF would also play an important role in BER in vivo.

SWI/SNF was unable to efficiently promote BER on the variant H2A.Bbd nucleosome. In this case, since a very low level of remodeling was occurring, efficient BER would require eviction of the variant H2A.Bbd-H2B dimer or of the histone octamer. Indeed, the H2A.Bbd nucleosome shows an easier spontaneous transfer of the H2A.Bbd-H2B dimer to an acceptor H3-H4 tetramer particle (3) and a weaker thermodynamic stability. H2A.Bbd was also found localized in active chromatin, where eviction of histones is known to take place (8).

The schematics depicted in Fig. 7 summarize our data on the role of SWI/SNF in BER on conventional nucleosomal templates. SWI/SNF assists BER by either remodeling or combined remodeling and sliding mechanisms. Our finding that remodeling alone was sufficient to dramatically increase the BER efficiency might have important implications for the mechanism of BER in vivo. Indeed, thousands of lesions are repaired by BER in a single eukaryotic cell per day, and the repair of these lesions has to be done constantly and very rapidly. Efficient BER without nucleosome sliding might be of crucial importance for the velocity of BER while maintaining the nucleosome positions.

We hypothesize that once the 8-oxoG is repaired, the remodeled nucleosome should return to its initial native state, either spontaneously due to thermal fluctuations or with the help of histone chaperones (Fig. 7). The latter is supported by data on CAF-1 (20) showing that CAF-1 is recruited to sites of UV lesion repair in vivo (reviewed in references 19, 36, and 40). Interestingly, the presence of histone chaperone activity accelerates nucleosome remodeling by SWI/SNF (1), which could be relevant to repair.

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